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# BIOLOGICAL BULLETIN

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## STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS.

### I., DATA ON THE SEXES.<sup>1</sup>

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#### I. INTRODUCTION.

According to Marchal ('04) and Silvestri ('06, '08) sex-determination in the polyembryonic chalcids is brought about in the same manner as in the bee. It is well known that the fertilized egg of the bee produces a female (queen or worker), while the unfertilized egg gives rise to a male (drone). Marchal and Silvestri believe that the fertilized egg of these parasitic hymenoptera produces a polyembryonic brood of females, while the unfertilized egg develops into a brood of males. This conclusion has been widely accepted, not only because it is in harmony with the Dzierzon theory of sex-determination in the bee, but also because several different investigators have observed that a polyembryonic brood usually does not contain individuals of both sexes. The occasional appearance of a mixed brood has been explained by the obvious and apparently logical assumption that a fertilized and an unfertilized egg had been deposited in a single egg of the host. The simultaneous development of two such eggs would naturally produce a mixed brood of parasites.

In a recent paper dealing with the development of the polyembryonic chalcid, *Copidosoma gelechiæ*, the writer ('15) called attention to the fact that it was difficult to explain the origin of certain mixed broods of this species on the assumption that two parasitic eggs carrying the opposite sex factors had been

<sup>1</sup> Contributions from the Zoölogical Laboratory of the University of Texas, No. 130.

deposited in the egg of the host. It was suggested that of the nine complete, mixed broods listed, the origin of only five (broods 2, 3, 4, 6, 7, Table I.) could easily be explained in this obvious way. In each of the four remaining broods (broods 1, 5, 8, 9) the great preponderance of females seemed to indicate that such an explanation was not tenable. It was therefore suggested that these mixed broods could have arisen from a single fertilized egg, through a differential division of the two sex chromosomes at one of the early divisions of the egg. In that connection the following statement was made: "If *Copidosoma* conforms to the general scheme for sex-determination in insects, the females must have the 2 X chromosomes, and males the single X chromosome. Ordinarily, during the process of cleavage, all of the chromosomes in the fertilized egg divide equally, so that all of the nuclei entering into the formation of the embryos will carry the XX chromosomes, thus producing a brood of females. But if during the early development of the egg it should happen that the two X chromosomes in one or more cleavages should not divide but separate, one going to each pole of the spindle, each daughter nucleus would then receive a single X chromosome. If such nuclei later divide in the typical manner and give rise to embryos, such embryos would be males."<sup>1</sup>

Different workers have from time to time offered a similar explanation to account for certain peculiarities in sex heredity, and especially to account for the origin of hermaphrodites and gynandromorphs. In doing so they have recognized the possibility that a qualitative division of the hereditary material for the sex factor may take place during cleavage. Their statements refer of course to the development of a monembryonic egg, in which the products of any somatic segregation must necessarily be distributed to different parts of the same embryo. In the case of a polyembryonic egg, a similar segregation, if occurring early enough in cleavage, would result in the production of two groups of embryos of different sexuality, and upon completing their development these two groups of embryos would produce a mixed brood.

It was this kind of evidence, although meager in amount,

<sup>1</sup> *Loc. cit.*, p. 356.

that was discovered in studying the broods of *Copidosoma*. Only four out of a total of 162 broods gave evidence suggesting that a differential division might have occurred during the segmentation of the egg. It was therefore evident that if such differential divisions do occur in these parasitic insects, it must be studied in a species in which the polyembryonic brood is much larger than that of *Copidosoma*. For it is clear that the larger the brood the greater must be the number of cell divisions in that period of development at the close of which each blastomere becomes the progenitor of an embryo, and consequently, the greater the opportunity for a differential division to occur,

## II. DATA ON SEXES OF PARACOPIDOSOMOPSIS.

At Austin such a species is found in *Paracopidosomopsis floridanus* Ashmead, a form very similar to the European chalcid, *Litomastix truncatellus*, upon which Silvestri worked. This

TABLE I.

MIXED BROODS OF *Copidosoma*.

Brood.	No. of Individuals.	Females.	Males.
1	162	153	9
2	172	92	80
3	207	126	81
4	216	176	40
5	235	223	12
6	241	161	80
7	300	235	65
8	304	292	12
9	337	316	21

little parasite lays its egg in the egg of the common cabbage looper, *Autographra brassicæ*. This species is very favorable for study, and can be reared in the laboratory. Upon consuming the contents of the *Autographra* larva, the larval parasites pupate within the skin of the host, forming the typical mummified carcass. During the last two seasons about two hundred carcasses have been collected from the field. The silken cocoons were removed from these, after which each was placed in a vial, which was closed with a loose plug of cotton. Under these conditions the parasites readily emerge.

One hundred and twenty-nine of these broods, taken at random,

have been studied in detail, with the result that a number of interesting facts concerning the sexes have been brought to light. Of the hundred and twenty-nine broods, three are pure female broods, sixteen pure male, and one hundred and ten mixed. The three female broods have 1,089, 1,306, and 1,859 individuals, respectively; while the sixteen male broods run as follows: 385, 432, 525, 578, 675, 699, 988, 1,003, 1,124, 1,277, 1,285, 1,288, 1,333, 1,354, 1,492, 1,588. This is an average of 1,001 individuals per brood.

It is the mixed broods which are of special interest. The number of individuals in these broods varies from 545 to 2,028, with an average of 1,246 individuals per brood. The percentage of males varies from 72.07 to .06. In Table II. are listed sixty broods, in which the sex of each individual has been carefully determined. These are arranged in the order of per cent. of males, from the highest to the lowest. Fifty additional broods are listed in Table III., in which the total number of individuals is given, and the per cent. of males as determined by a study of five hundred individuals in each brood. This method of studying the sexes does not give the exact number of males and females in a brood, but it yields a very close approximation to the true per cent. of males.

These data raise a number of interesting points, only a few of which can be considered here. One of the most striking features is the scarcity of pure broods. Of all the broods studied, only about twelve per cent. are male broods, and less than three per cent. female broods. Another equally striking feature is the great preponderance of females in the mixed broods. In only seven of the one hundred and ten such broods is the number of males in excess of the number of females (broods 1, 2, 3, 4, 5, 6, 61, Tables II., III.). In the remaining one hundred and three cases the females are in the majority.

Reference to Tables II. and III. will show that in over 58 per cent. of the broods less than ten per cent. of the individuals in any given brood are males, while in 35 per cent. of the cases there is less than three per cent. of males. The most striking cases are those listed toward the bottom of the tables, especially the last six or seven broods in each table. In each of the last

TABLE II.

MIXED BROODS OF *Paracopidosomopsis*.

Brood.	No. of Individuals.	Females.	Males.	Per Cent. of Ma
1	974	272	702	72.07
2	1,289	400	889	68.97
3	1,237	482	755	61.02
4	636	294	342	53.78
5	828	385	443	53.50
6	545	272	273	50.09
7	1,162	595	565	48.71
8	848	529	319	37.62
9	1,512	1,009	503	33.27
10	1,847	1,287	560	30.32
11	839	586	253	30.15
12	943	701	242	25.66
13	998	746	252	25.25
14	1,706	1,353	353	20.69
15	628	509	119	18.95
16	662	542	120	18.12
17	1,022	878	144	14.09
18	1,364	1,195	169	12.39
19	1,797	1,584	213	11.85
20	1,041	923	118	11.35
21	1,477	1,357	120	8.12
22	1,115	1,033	82	7.35
23	1,307	1,211	96	7.35
24	1,303	1,199	104	7.21
25	1,196	1,122	74	6.19
26	803	754	49	6.10
27	1,432	1,352	80	5.59
28	868	822	46	5.30
29	1,423	1,349	74	5.20
30	1,050	996	54	5.14
31	1,883	1,791	92	4.89
32	1,259	1,203	56	4.45
33	1,457	1,394	63	4.32
34	855	828	27	3.16
35	1,046	1,015	31	2.96
36	1,124	1,091	33	2.94
37	1,000	972	28	2.80
38	757	739	18	2.38
39	847	827	20	2.36
40	1,301	1,271	30	2.31
41	1,209	1,183	26	2.15
42	1,260	1,233	27	2.14
43	1,682	1,647	35	2.08
44	1,592	1,559	33	2.07
45	1,258	1,232	26	2.07
46	692	679	13	1.88
47	1,220	1,200	20	1.64
48	1,115	1,097	18	1.61
49	1,036	1,021	15	1.46
50	1,798	1,774	24	1.35
51	1,075	1,061	14	1.30
52	1,475	1,456	19	1.29
53	1,349	1,336	13	.96
54	2,003	1,986	17	.85
55	1,017	1,010	7	.69

TABLE II.—*Continued.*  
MIXED BROODS OF *Paracopidosomopsis*.

Brood:	No. of Individuals.	Females.	Males.	Per Cent of Males.
56	1,165	1,159	6	.52
57	1,143	1,141	2	.18
58	1,493	1,491	2	.13
59	912	911	1	.12
60	1,550	1,549	1	.06

two broods of Table II. only a single male was present. *In contrast with this condition, broods with a large number of males and a few females have so far not been found.* As stated above, in only seven cases out of a hundred and ten are males in excess

TABLE III.  
MIXED BROODS OF *Paracopidosomopsis*.  
Per Cent. of Males Based on a Study of 500 Individuals.

Brood.	No. of Individuals.	Per Cent. of Males.	Brood.	No. of Individuals.	Per Cent. of Males.
61	2,028	67.20	86	701	11.00
62	1,043	47.50	87	1,403	9.20
63	1,149	44.00	88	1,602	8.20
64	1,540	43.90	89	1,606	8.09
65	1,463	42.80	90	1,228	8.00
66	1,134	37.00	91	1,614	7.40
67	1,397	31.60	92	1,675	7.40
68	940	31.20	93	1,258	6.50
69	1,123	29.60	94	1,139	6.00
70	996	29.00	95	841	5.20
71	1,045	26.00	96	1,111	4.40
72	1,467	24.80	97	1,665	3.40
73	1,822	24.00	98	1,351	2.80
74	1,233	22.60	99	1,633	2.80
75	1,685	21.80	100	1,857	2.40
76	1,095	19.60	101	794	2.20
77	1,745	19.00	102	1,306	2.20
78	1,201	18.00	103	1,092	2.00
79	1,301	16.00	104	980	2.00
80	841	14.60	105	1,005	1.80
81	1,410	14.60	106	1,334	1.20
82	1,605	14.20	107	1,550	1.20
83	867	13.60	108	1,560	1.00
84	1,347	12.20	109	1,469	.60
85	1,167	11.80	110	1,227	.60

of females, and even in these the smallest number of females is almost as much as 28 per cent. of the entire brood (brood 1). The fact that females are so frequently in excess of males in the broods is one of the most significant points brought to light in the study of these parasites.

## III. SUGGESTED EXPLANATIONS OF MIXED BROODS.

On the basis of the data here presented, how is one to explain the origin of mixed broods in *Paracopidosomopsis*? There are at least two possible explanations that may be suggested: (1) that a fertilized and an unfertilized egg had been deposited in the egg of the host; (2) that a fertilized egg had given rise to males as well as to females.

One finds no difficulty in offering the first of these suggested explanations to account for some of the mixed broods, especially for the larger broods in which males and females are found in about equal numbers. But if the same explanation is to be applied to all mixed broods, it would be necessary to assume that in the majority of cases the development of the unfertilized egg is interfered with by the presence of the developing fertilized egg. In the light of Lillie's ('16) recent discovery concerning the origin of the "free martin" in cattle, this assumption would not seem to be untenable. In the case of these insects it is conceivable that a fertilized egg might generate "hormones" which would inhibit the development of an unfertilized egg in such a way that only a few of the male embryos reach maturity.

One difficulty which at first seemed to stand in the way of the application of the hormone theory is the fact that the vast majority (about 85 per cent.) of all *Paracopidosomopsis* broods are mixed. It did not seem possible that eighty-five per cent. of the moth eggs, widely scattered as they are in the cabbage patch, would each be visited and parasitized by a fertilized and an unfertilized female insect. This raised the question as to whether the female lays more than a single egg at each oviposition, and if so, whether the fertilized female has the power to lay both kinds of eggs, as is the case of the queen bee. Silvestri states that the female of *Litomastix truncatellus* lays but a single egg at each oviposition. We have studied this question in *Paracopidosomopsis* and find that the female, whether fertilized or not, frequently deposits two eggs at one oviposition. In one experiment, a single unfertilized female made seventeen successive ovipositions in as many eggs. The time of oviposition varied from one minute and thirty seconds to three minutes, with an average of two minutes for each egg. An



examination of these parasitized eggs in carefully teased preparations showed that the parasite had laid two eggs in eleven cases, and a single egg in six cases. The same experiment performed with fertilized females shows the same result. In a total of forty-two ovipositions made by three unfertilized and sixteen fertilized females, two eggs were laid in twenty-eight cases and one egg in fourteen cases.

While these observations show conclusively that two eggs are frequently deposited simultaneously by the insect, yet they do not settle the question as to whether the fertilized female can lay both kinds of eggs. However, a study of smear preparations and sections shows that in many cases both eggs are fertilized. For this, as well as for other reasons, the writer is of the opinion that any suggested explanation involving the idea that an unfertilized and a fertilized egg is laid in the egg of the host is inadequate to account for all of the mixed broods in *Paracopidosomopsis*.

This brings us to a consideration of the second suggestion, namely, that a fertilized egg may give rise to males and females. This result could be attained by the abnormal behavior of the sex chromosomes in one or more of the cleavage divisions. There are several different ways in which an abnormal behavior of the sex chromosomes might occur. Some of these possibilities are too improbable to receive consideration, but there are three or four that we shall mention. First, by *somatic disjunction*,<sup>1</sup> by which is meant the separation instead of division of the two sex chromosomes in a cleavage mitosis. Second, by *somatic non-disjunction*, by which is meant the failure of the two daughter chromosomes of one of the X's to separate after the division of both sex chromosomes had occurred. This would result in one daughter cell receiving three sex chromosomes, the other a single X chromosome. A third possibility is that only one X chromosome might divide, resulting in the formation of one cell with two X's and another with only one X. Finally, we may mention a fourth possibility, namely, that a division might occur in which all the sex chromosomes, whether divided or not, pass

<sup>1</sup> This term is not entirely exact, because of the implication that the separating chromosomes had previously been united, which of course is not the case.

to one pole of the spindle. In this event one of the daughter cells would contain no sex chromosome. Let us consider further each of these possibilities.

The mechanism of a disjunctional division was explained in connection with the study of *Copidosoma*, from which a quotation is given in the first part of this paper. It remains therefore to point out what would happen should such a differential division occur. If a disjunctional division should occur during cleavage, the percentage of males to females would be determined by the time at which the differential division occurs. For example, if disjunction took place at the first cleavage of the fertilized egg, each of the two daughter cells would receive a single X chromosome, and should no further disjunctions occur in subsequent divisions, a pure male brood would be produced. If on the same basis the disjunction did not occur until the second division, only fifty per cent. of the brood would be males; if at the third, twenty-five per cent.; if at the fourth, twelve and one half per cent., and so on decreasingly until the point is reached at which each cell becomes the progenitor of a single embryo. However, in any random lot of mixed broods these expected classes, based on per cent. of males would not be realized, because there are certain factors in development which would tend to modify the expected percentage of males in any given brood. Thus not all of the cells divide simultaneously in the early cleavages of the egg of these parasites. Some cells may fail to develop altogether. More than one disjunctional division may occur in the egg. Finally, some cells develop into the so-called assexual larvæ, which never reach maturity. In a random lot of broods we should therefore expect to find a continuous series, such as is shown in the tables.

Of the several possibilities suggested that of somatic non-disjunction would seem to be the most plausible. First, because it is in harmony with the known facts of cytology on cleavage mitosis, in that all of the chromosomes are supposed to divide; and second, because it is in accord with Bridges' ('16) work on non-disjunction in *Drosophila*. This investigator has recently suggested the possibility of somatic non-disjunction to account for gynandromorphs and mosaics in a monembryonic egg,

such as that of *Drosophila*. He says: "If the same sort of primary non-disjunction which has been assumed to give rise to XX'X' cells in an XX female, should take place at a cleavage stage, gynandromorphs and mosaics would result. One might expect at an early cleavage division, particularly the first, a relatively large number of X-XX'X' divisions, for the greatly condensed chromosomes introduced by the sperm do not for some time attain the state or the appearance of those of the egg nucleus. If the paternal X of a female were slower than the maternal in preparing for division, it might lag upon the spindle so that both daughter X's would become included in the same cell. The portion of the fly which comes from the X cell should be male and should show the sex-linked characters of the mother. Such a process may be the explanation of the large number of lateral gynandromorphs of *Drosophila*. When the X-XX'X' division occurs at a later cleavage stage we may have mosaics, as for example, a red female with a patch of white facets in the eye."<sup>1</sup>

In non-disjunction the per cent. of males in any brood would be determined, as in disjunction, by the time at which the division takes place. However, there would be this difference, that should the first cleavage be non-disjunctional, the expected percentage of males would be fifty instead of one hundred; should the second cleavage be non-disjunctional, twenty-five per cent. would be males instead of fifty, and so on, decreasingly throughout the series of cleavage divisions.

The third possibility mentioned above is in reality only a modified form of non-disjunction, and the percentage of males would be determined in accordance with the same law.

The fourth possibility suggested above is of interest because such a division would result in the production of blastomeres devoid of sex chromosomes. Cells which contain no X chromosomes may be responsible for the so-called asexual larvæ that develop from the egg of this species. We shall consider this point further in connection with a discussion of these peculiar larvæ.

<sup>1</sup> *Loc. cit.*, p. 136.

## IV. EXPERIMENTAL AND CYTOLOGICAL STUDIES.

If any one of these forms of abnormal behavior of sex chromosomes does occur in the cleavage divisions of *Paracopidosomopsis*, it should be possible to detect it by experimental and cytological studies. We have undertaken both lines of investigation, and already sufficient progress has been made to clear up certain points.

The experimental work has been greatly interfered with by the ravages of the so-called wilt disease, to which the *Autographa* larvæ are extremely susceptible under laboratory conditions. In all of the experimental work the precaution has been taken to protect the moth eggs from being parasitized before beginning any experiment with them. This has been done by having the moth lay its eggs under a bell-jar from which all parasites were scrupulously excluded. Furthermore, the parasitized eggs and the young larvæ developing from them have been similarly protected. In all experiments the female parasite has been permitted to make but one oviposition in each moth egg.

The first set of experiments were undertaken with a view to determine whether the fertilized female parasite could lay eggs which produce males and females. In one experiment 23 moth eggs were parasitized by fertilized females. Sixteen larvæ hatched three days later. Of these two reached full growth. One of the two larvæ was not parasitized, and formed a pupa from which a moth later emerged. The other was transformed into a typical mummified carcass, from which a brood of parasites in due time emerged. The brood contained 2,096 individuals, of which 1,889 were females and 207 males. While these results are meager, nevertheless they indicate clearly that a fertilized female is capable of laying eggs from which individuals of both sexes develop. In this instance it is not possible to tell whether the mixed brood came from one egg or from two eggs, since there was no way of determining the number of eggs deposited by the parasite in the egg of the host at the time she made the oviposition.

In another set of experiments unfertilized females were used. In one experiment eighty moth eggs were parasitized, each by one oviposition by an unfertilized female. Sixty-six larvæ

hatched. All but twenty-two of these larvæ were fixed at various stages of development, in order to secure material for the study of the unfertilized parasitic egg. Three of the twenty-two caterpillars eventually reach full or nearly full growth. Two of these showed signs of disease, and were killed, the parasitic larvæ being removed and fixed for cytological study. A study of sections of the larvæ from both caterpillars fail to show anything but male larvæ present. The third caterpillar formed a carcass, from which 1,842 males emerged. To this must be added 100 more individuals, which were removed from the carcass in the pupa stage and fixed for cytological purposes. The sections show that all the pupa are males. These results indicate that the unfertilized egg of *Paracopidosomopsis* produces males only.

A study of the spermatogenesis as revealed in these pupæ shows that males developed by parthenogenesis carry the haploid number of chromosomes, while a study of maturation of the eggs and fertilization shows that females, as would be expected, carry the diploid number. With these facts established, it will be possible to determine whether two types of males are produced, as may be shown by the following consideration. If *Paracopidosomopsis* conforms to the general scheme of sex determination in insects, the chromosomal formula for the fertilized egg may be represented by  $N + 2X$ , in which  $N$  represents the non-sex chromosomes, and that for the unfertilized egg by  $\frac{N}{2} + X$ . Now, should males also arise from the fertilized egg, their formula must be  $N + X$ . The presence of the double number of non-sex chromosomes in such individuals should not affect their sexuality, because in all forms in which parthenogenesis is absent, males are represented by this or a slightly modified formula. Modern genetic studies have shown that maleness or femaleness is determined by the number of X chromosomes present in the egg, and not by the number of non-sex chromosomes. Even in forms which have the Y chromosome, the presence or absence of the Y does not seem to affect the sex of the individuals.

## V. THE ASEXUAL LARVÆ.

Asexual larvæ were first described by Silvestri for *Litomastix*. According to this investigator, the egg of *Litomastix* produces a thousand or more sexual larvæ and a variable number of asexual larvæ, which are characterized by the absence of certain organs, and especially by the absence of the reproductive system. These larvæ die without undergoing metamorphosis. Exactly similar larvæ develop from the egg of *Paracopidosomopsis*. The details of their development have not as yet been studied, so that I cannot say whether they appear in every egg or not. These curious larvæ develop both in the fertilized and in the unfertilized egg, even though the host egg be rigidly protected from all other parasites. This shows that the asexual larvæ do not come from the egg of another parasitic species, as has been suggested as an explanation for their appearance among the larvæ of *Litomastix* (Wheeler, '10). An account of their development will be made the subject of a subsequent paper. Here, we are concerned with the question of the underlying cause of their production.

Silvestri suggests that these asexual larvæ may owe their asexuality to the absence of germ cells. It will be recalled that he described for the polyembryonic egg a so-called nucleolus (nucleolo), which lies at the larger or posterior end of the elongated, bottle-shaped egg. In the early cleavage stages of *Litomastix* the nucleolus becomes included in a single blastomere, resulting in the retardation of the subsequent divisions of this cell. The nucleolus later breaks down and becomes scattered evenly throughout the cytoplasm. Silvestri was unable to follow the history of this particular cell beyond two divisions, but he believes that it gives rise to all the germ cells of the sexual embryos. His conclusion has been strengthened by a subsequent study of the eggs of two monembryonic parasites. The nucleolus in the egg of these species becomes distributed to the germ cells alone, and thus serves as a "keimbahn-determinant" (Hegner).

According to Silvestri's suggestion the asexual larvæ of *Litomastix* arise from the polygerm, but fail to receive germ cells or descendants of the single blastomere which inherited the nucleolus. It may be that this suggestion of Silvestri offers the correct

explanation of the origin of the asexual larvæ, but there are certain obstacles which stand in the way of its full acceptance. In the first place, it is difficult to conceive of any mechanism in the complex polygerm by which predestined germ cells could be nicely and exactly distributed to each of the several hundred sexual embryos which develop from the eggs of *Litomastix* and *Paracopidosomopsis*. In the second place, it is a well-known fact that in insects secondary sexual characters and also certain primary sexual characters, such as the organs of copulation and oviposition, are not dependent upon the presence of the gonads for their development. This conclusion is based upon trustworthy evidences obtained from castration experiments on young larval stages. Furthermore, in the light of modern genetic evidence, I should be inclined to believe that the asexual larvæ are sexless not because they have failed to inherit predestined germ cells, but because of their failure to inherit X chromosomes. If X-free blastomeres are formed during cleavage, such cells might become the progenitors of sexless larvæ, which in a way could be compared to the non-viable, OY zygote of *Drosophila*.

#### VI. DATA ON SEXES OF OTHER POLYEMBRYONIC INSECTS.

With the exception of *Copidosoma gelechiæ* and *Paracopidosomopsis*, exact data on the sexes of polyembryonic insects are very few. The first observations recorded are those of Bugnion ('91), who studied twenty-one cases of *Ageniaspis* (*Encyrtus*) *fuscicollis* and found the following conditions: nine pure female broods; five pure male broods; three broods with males and females in about equal numbers; three broods with large majority of males; one brood with large majority of females. In commenting upon these data, Marchal ('04) points out that it is difficult to explain the four cases in which the large majority of individuals belong to one or the other sex. He believes, however, that such broods arise from two or more eggs. He further believes that should two eggs, one fertilized and the other not, be laid in the egg at slightly different times, the one deposited first might gain the upper hand in the matter of food, and thus prevent all but a few of the individuals arising from the second egg from reaching maturity.

Marchal examined sixteen broods of *Polygnotus minutus*, and found eight female broods, six male, and two mixed, one with three females and one male, the other with three females and three males.

The only other investigator to record data on the sexes of polyembryonic parasites is Silvestri. In one hundred and sixty broods of *Litomastix* he found sixty-three female, ninety-two male, and five mixed broods. He does not record the number of individuals for any of these broods.

I should like to point out in conclusion that there is very great need for exact data on the sexes of polyembryonic insects.

AUSTIN, TEXAS,

January 17, 1917.

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